

REPLACEMENT
SHEET
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STEPS IN
PCR/RE/LDR

WILD-TYPE

MUTANT

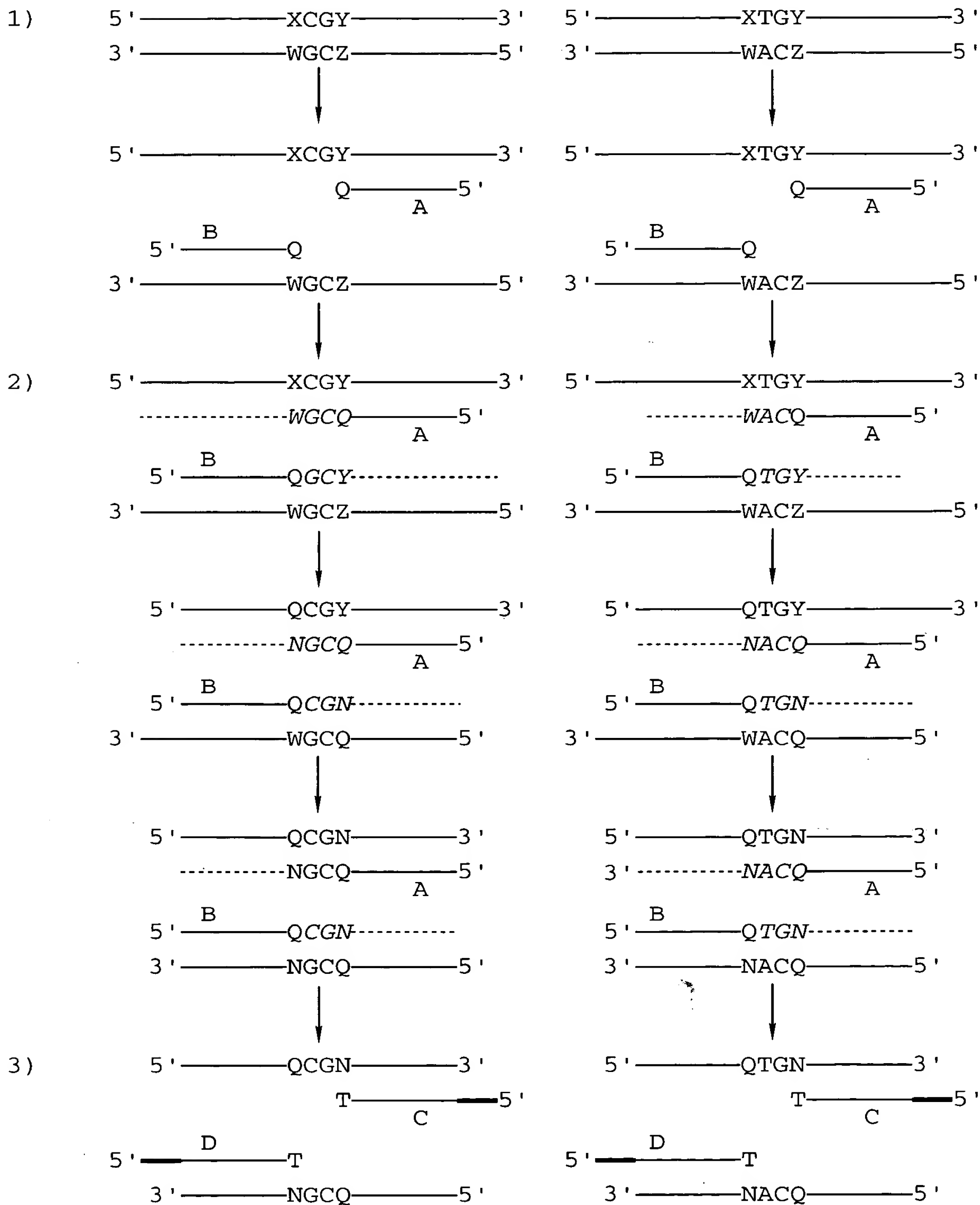
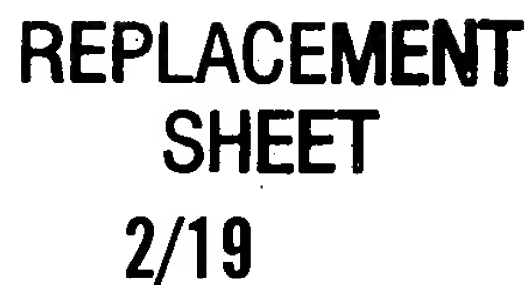


FIG. 1A





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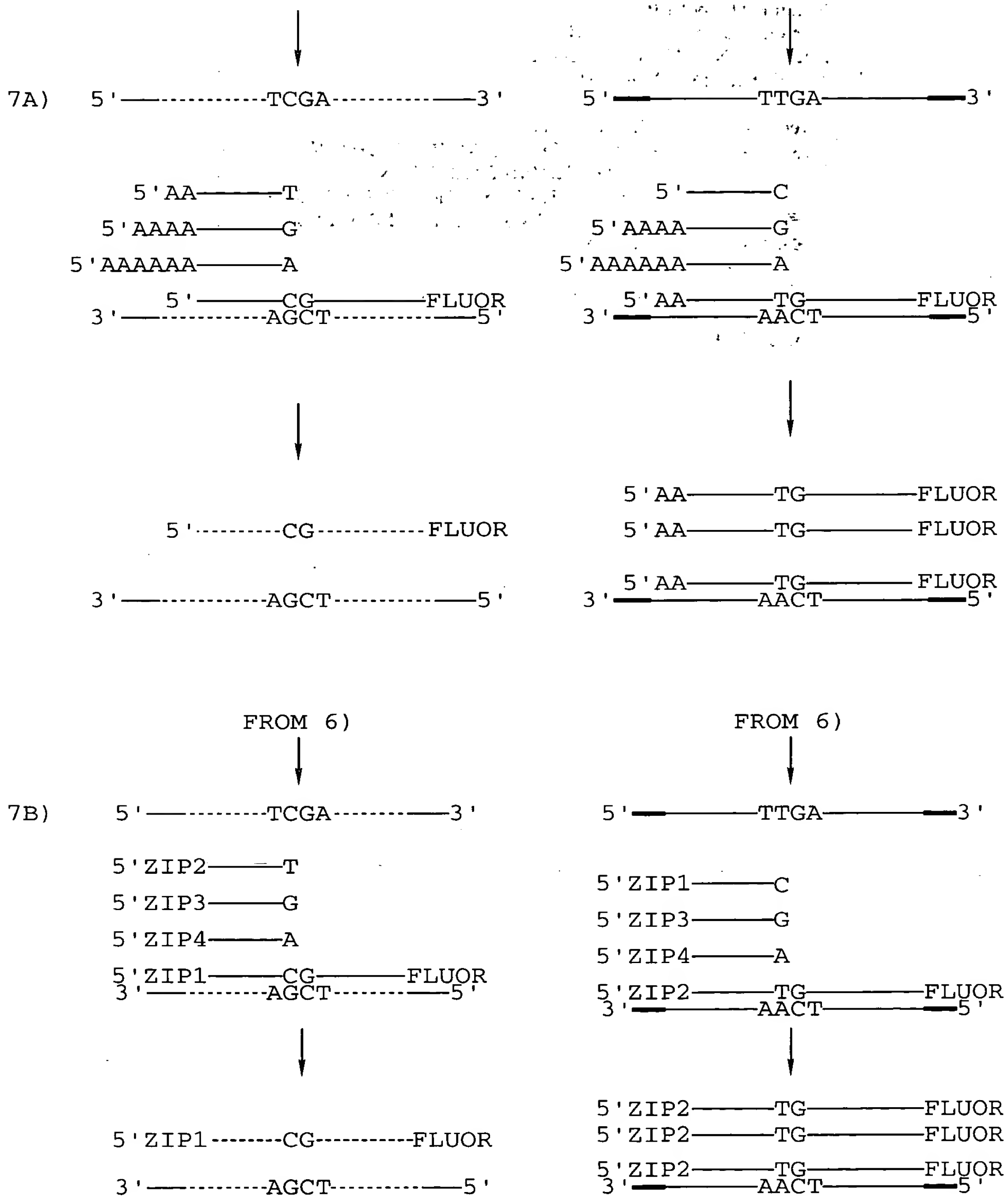


FIG. 1C



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5' AA ——— TG ——— FL

5' CG FL

5' AAAAAA ——— A

5' AAAA ——— G

5' G FL

5' AA ——— T

5' ——— C

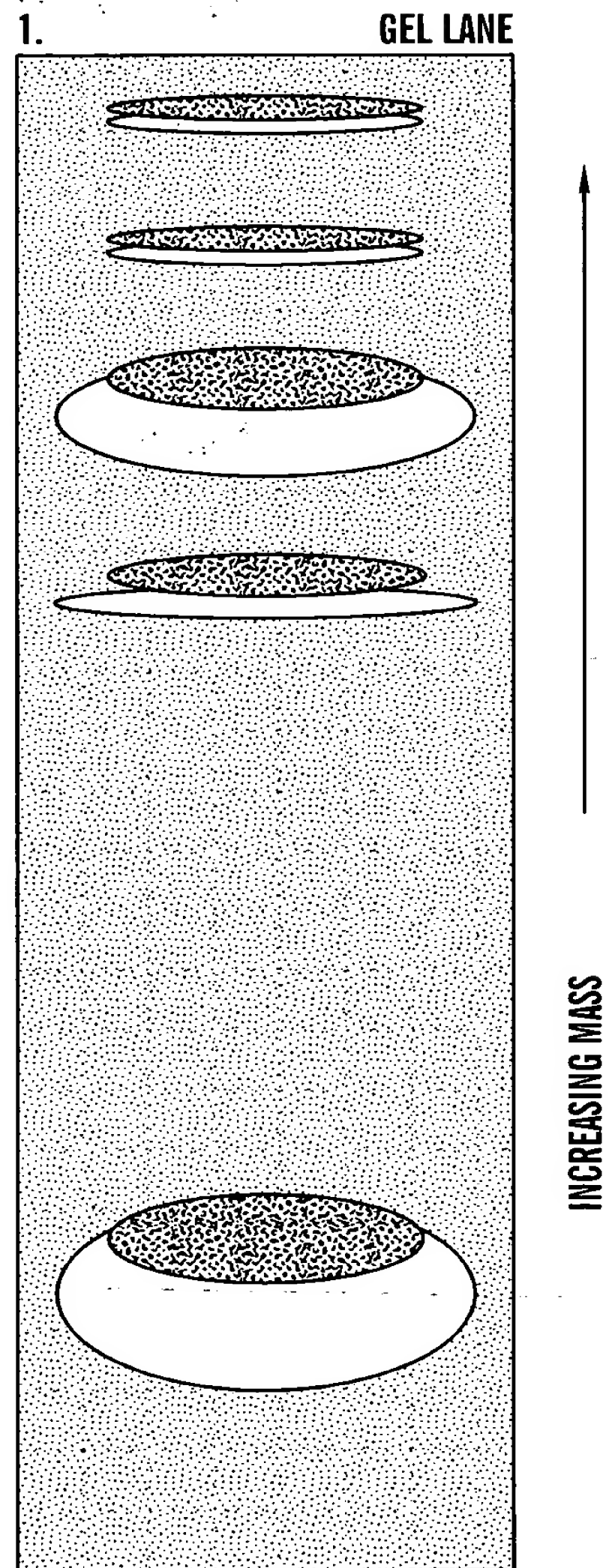


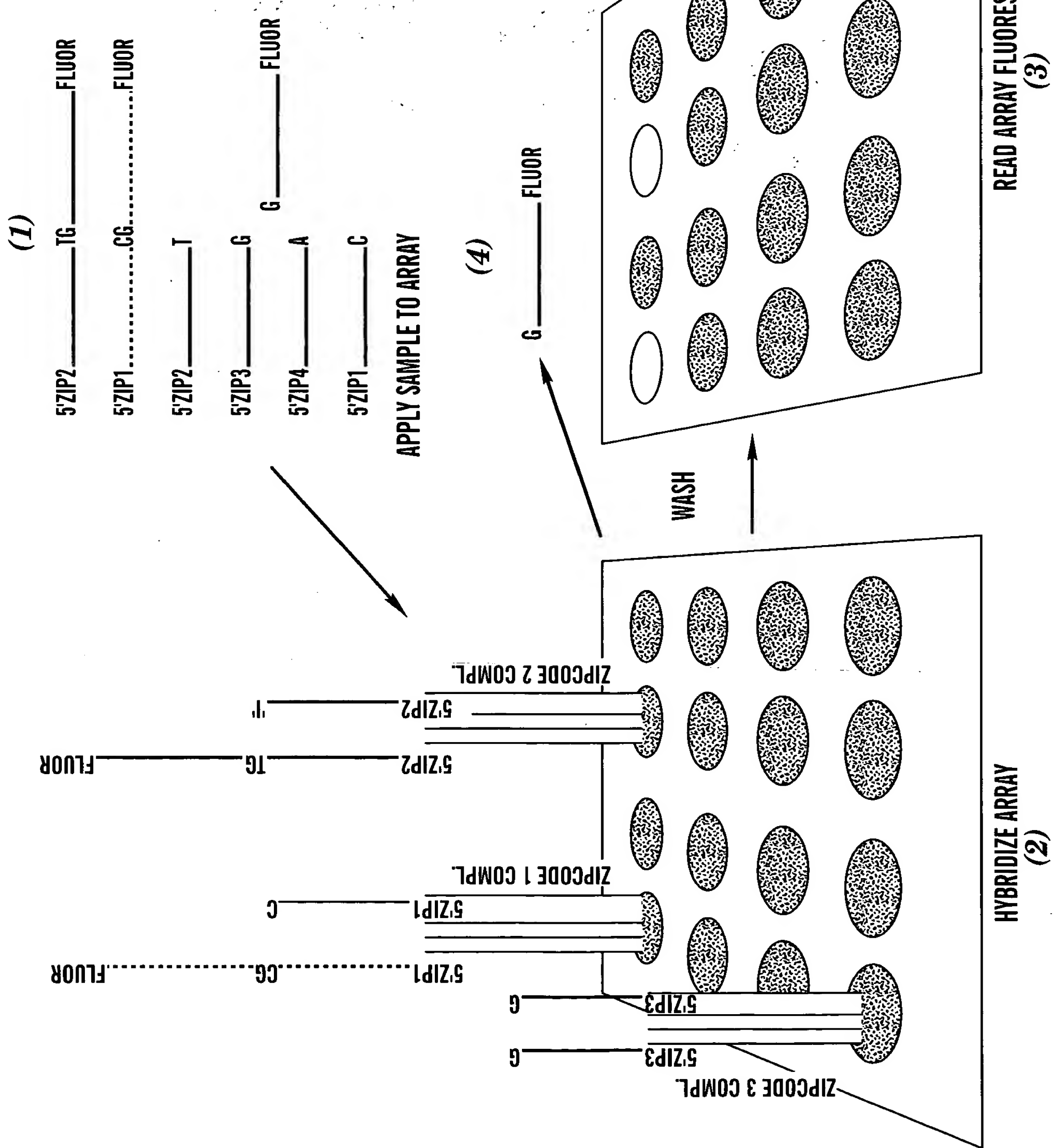
FIG. 2

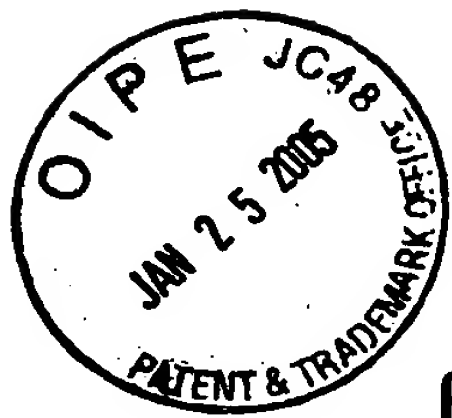


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FIG. 3





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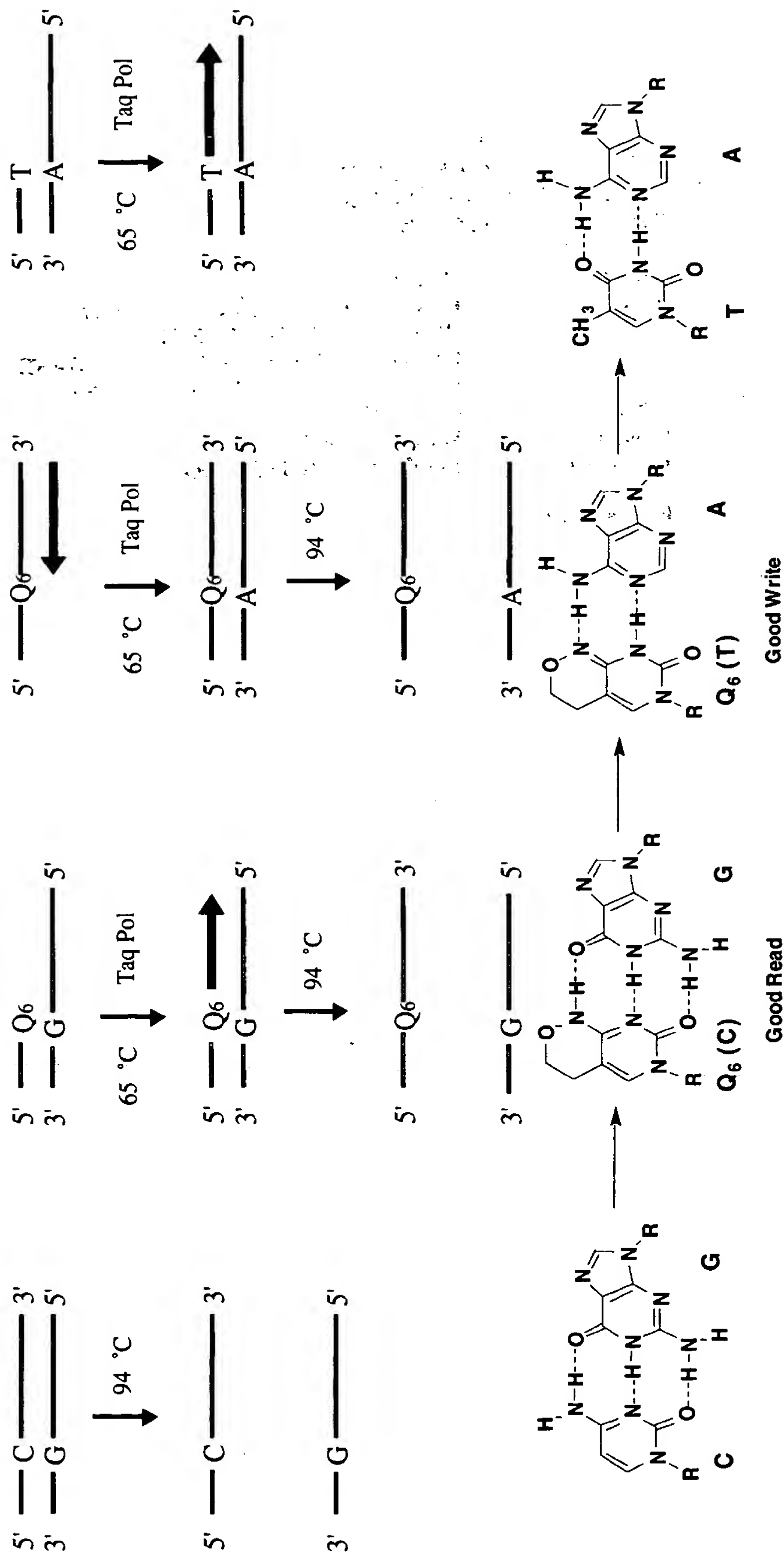
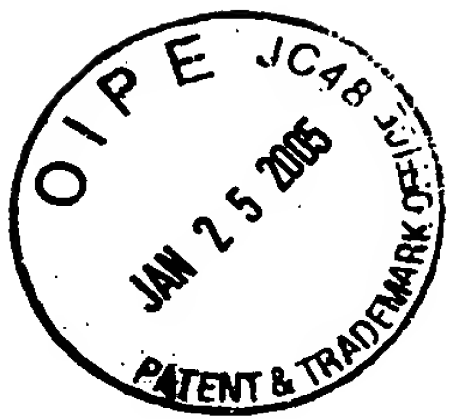


FIG. 4



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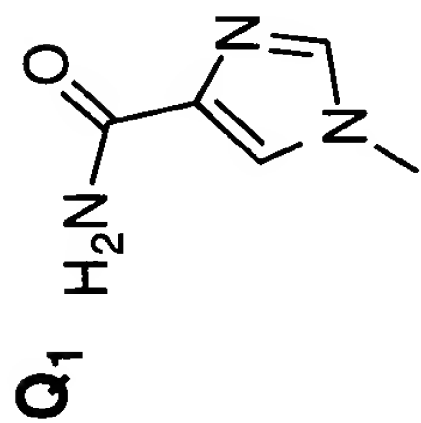


FIG. 5A

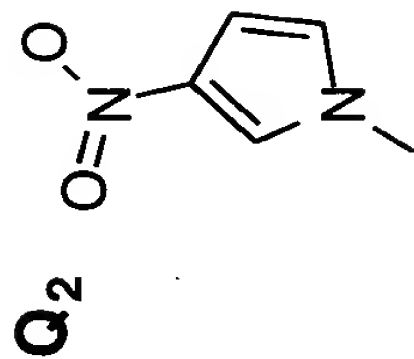


FIG. 5B

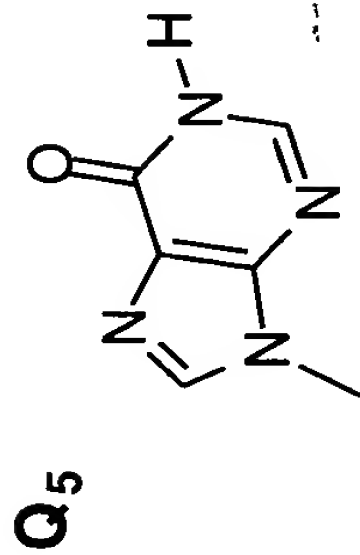


FIG. 5C

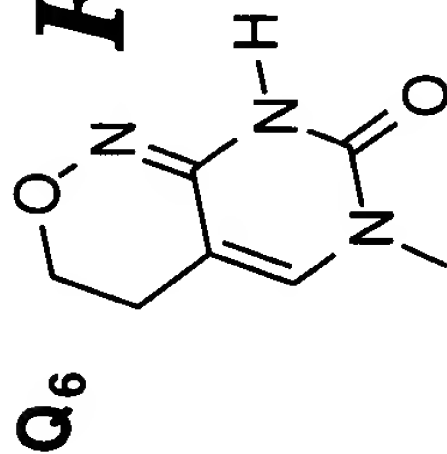


FIG. 5D

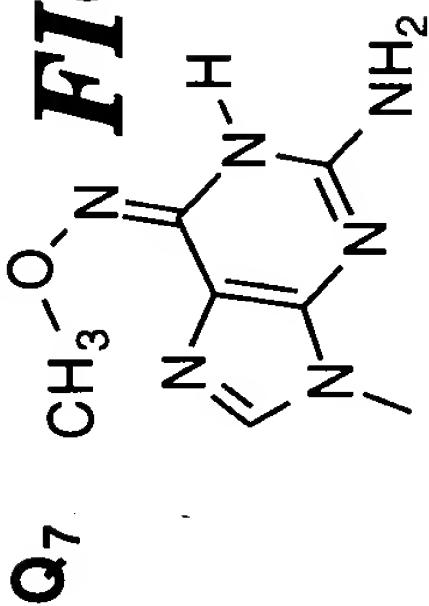


FIG. 5E

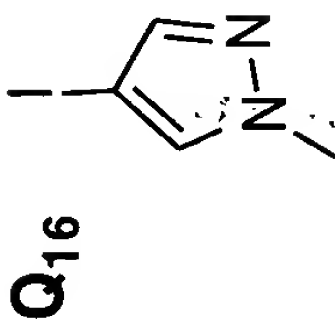


FIG. 5F

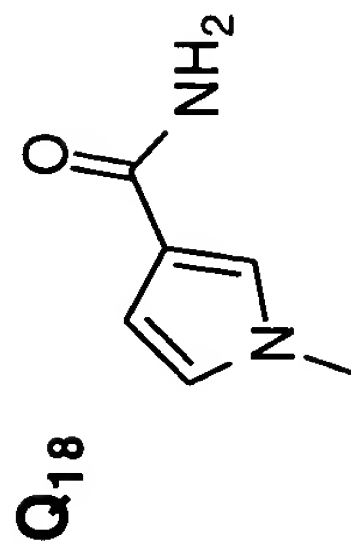


FIG. 5G

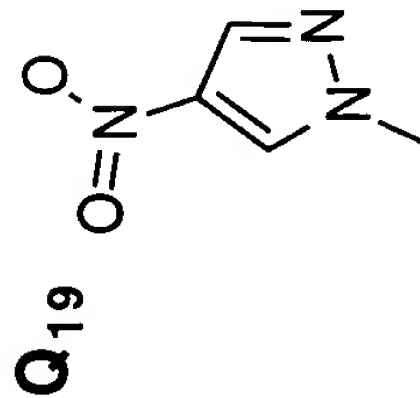


FIG. 5H

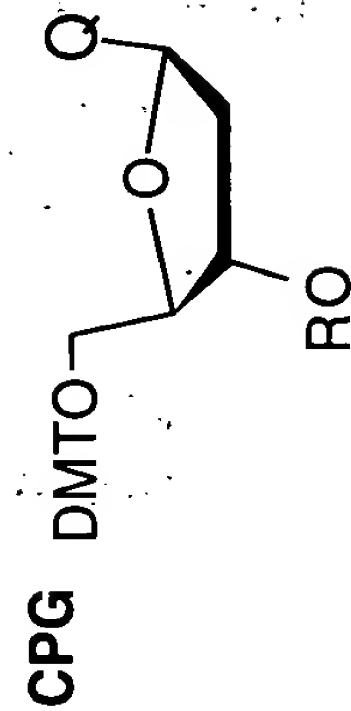


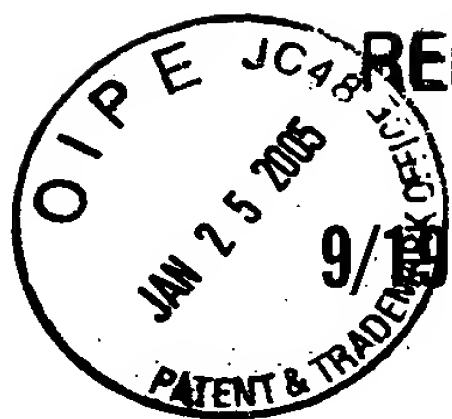
FIG. 5I



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codon248

FIG. 6A



REPLACEMENT SHEET

PRIMERS

codon248

Ztop CTT GGA CGA GTT CAT ACG C

P53zip248T CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG AAT

P53-248Q_N T TCT TCC TGC ATG GGC GGC ATG AAQ_N → pol
|| ||| ||| ||| ||| ||| ||| |||

50 bp synthetic 3' CA AGG AGC TAC CCG CCG TAC TTG GCC TCC GCG TAG GAG TGG TAG TAG TGT 5' (-)

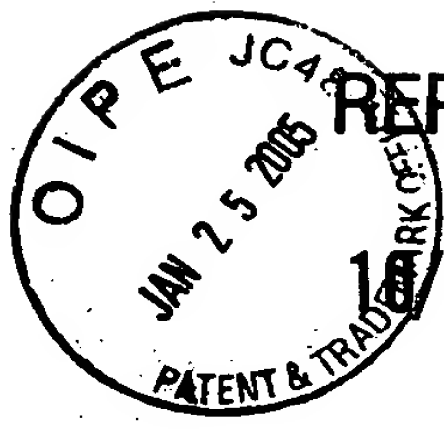
duplex DNA 5' GT TCC TGC ATG GGC GGC ATG AAC CGG AGG CCC ATC CTC ACC ATC ATC ACA 3' (+)
: ||| ||| ||| ||| ||| ||| ||| |||

p53-248 Q_N^R pol → Q_N TCC GCG TAG GAG TGG TAG TAG TCT T

p53zip248TR T TCC GCG TAG GAG TGG TAG TGC ACC GCT GGG TCA AAC G

Zbot C ACC GCT GGG TCA AAC G

FIG. 6B



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<u>LDR PRIMERS</u>		DISCRIMINATION										COMMON							
P53LDR248FCA	F-AAAAAA	GC	ATG	GGC	GGC	ATG	AAC	A											
P53LDR248FCG	F-AAAA	GC	ATG	GGC	GGC	ATG	AAC	G											
P53LDR248FCT	F-AA	GC	ATG	GGC	GGC	ATG	AAC	T											
P53LDR248FCC	F-	GC	ATG	GGC	GGC	ATG	AAC	C	... <i>ligase</i>										
P53LDR248PGG									GG	AGG	CCC	ATC	CTC	ACC	ATC	AT-block			
3' (- strand)																		5'	
CONVERSION	...GTA	TGC	GCA	AGG	ACG	TAC	CCG	CCG	TAC	TTG	NCC	TCC	GGG	TAG	GAG	TGG	TAG	TGA	ACC...
PRODUCTS																			

FIG. 6C



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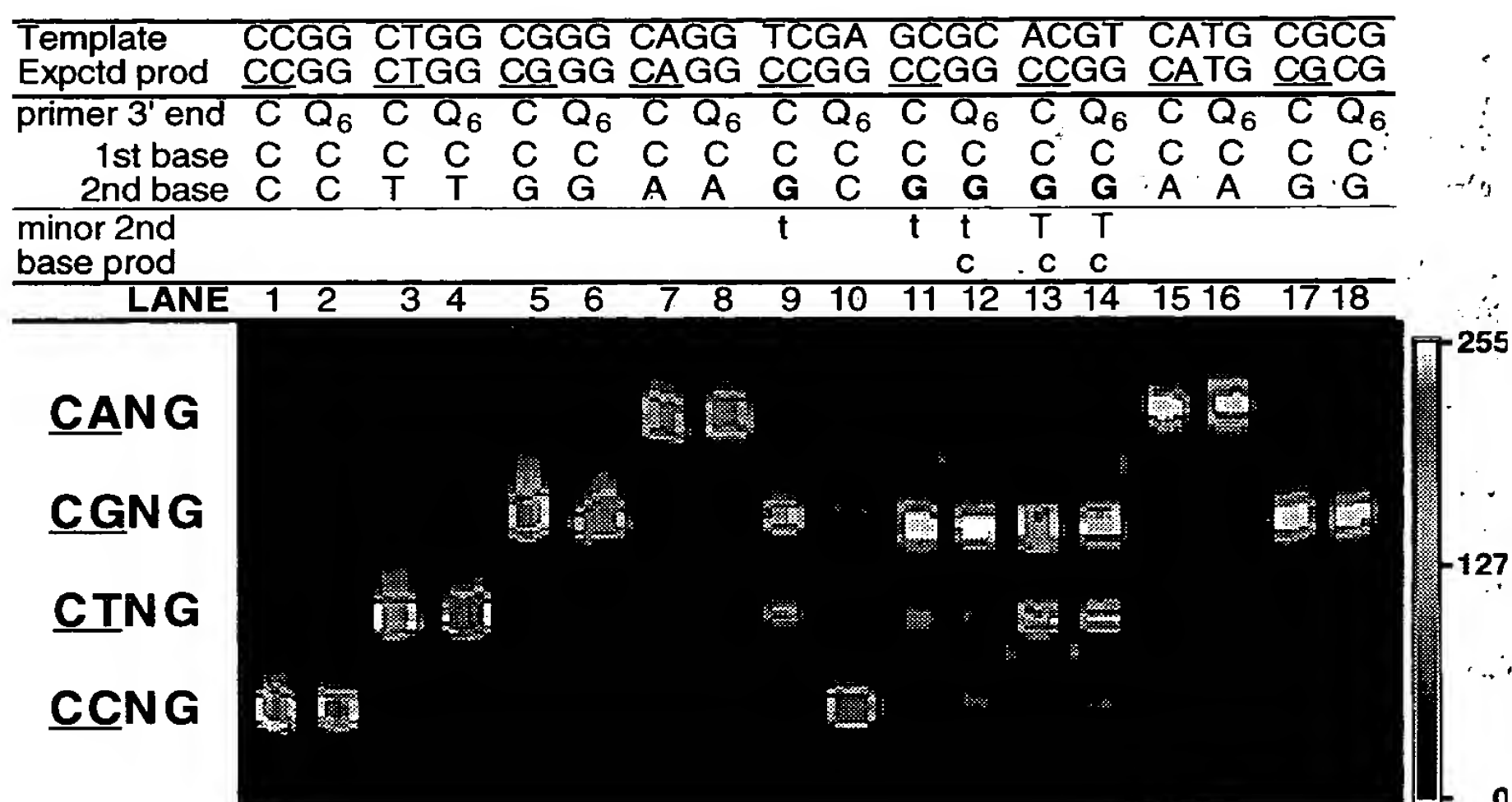
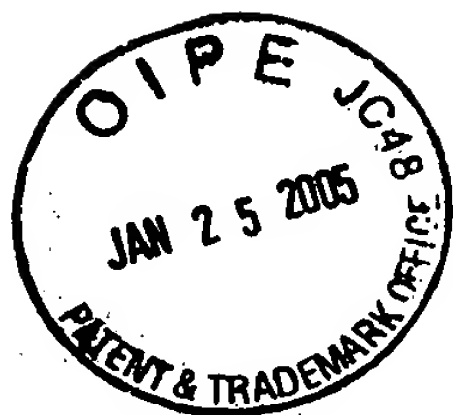


FIG. 7A



FIG. 7B



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Template	CCGG			CTGG			CGGG			CAGG			TCGA			GCGC			ACGT			CATG			CGCG			
Exptd prod	<u>GC</u> GC			<u>GT</u> GC			<u>GG</u> GC			<u>GAG</u> C			<u>GCG</u> C			<u>GCG</u> C			<u>GCG</u> C			<u>GAT</u> C			<u>GGC</u> C			
primer 3' end	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	G	Q ₅	Q ₇	
1st base	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G	G
2nd base	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C
minor 2nd base prod				t	t	t				g	g		A	A	a													
	LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27

GANC

GGNC

GTNC

GCNC

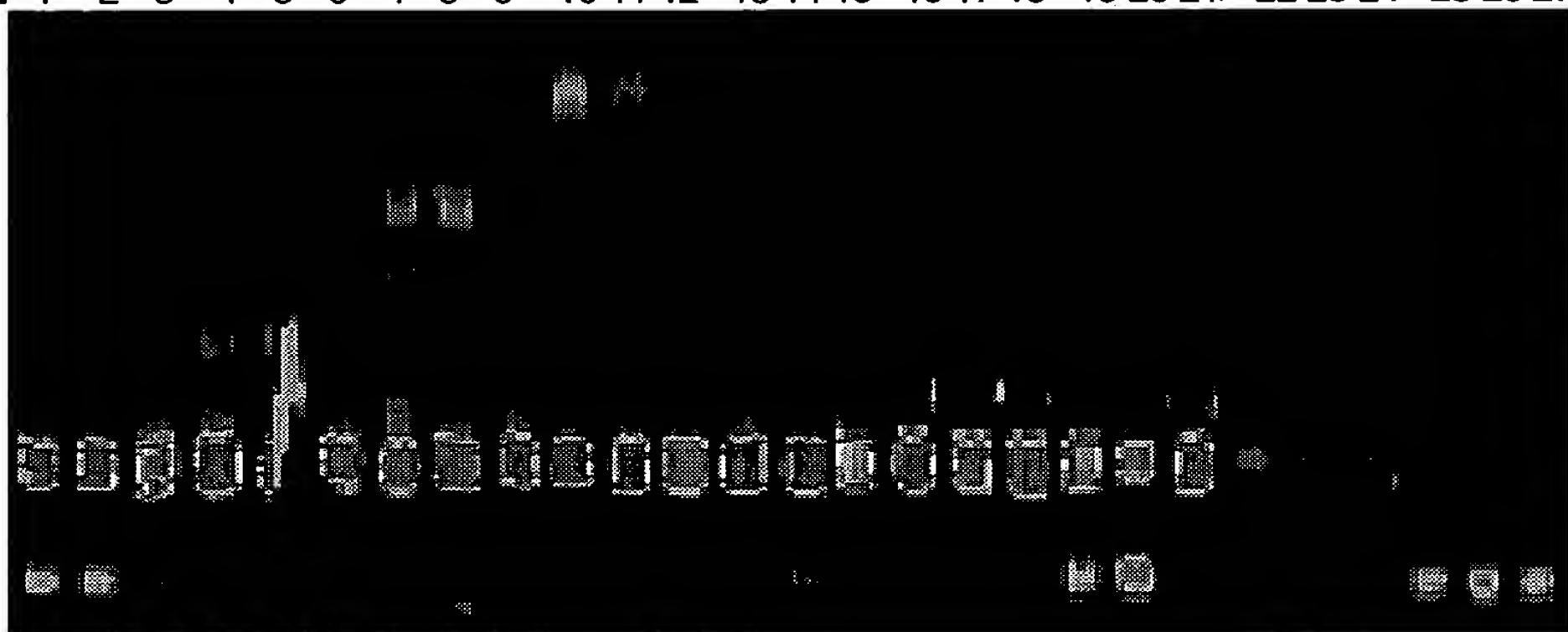


FIG. 8A

Template	CCGG			CTGG			CGGG			CAGG			TCGA			GCGC			ACGT			CATG			CGCG					
Exptd prod	<u>ACGT</u>			<u>ATGT</u>			<u>AGGT</u>			<u>AAGT</u>			<u>ACGT</u>			<u>ACGT</u>			<u>ACGT</u>			<u>AATT</u>			<u>AGCT</u>					
primer 3' end	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅	A	Q ₇	Q ₅			
1st base	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A		
2nd base	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C	C		
minor 2nd base prod	?						?																							
LANE	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27			

AANT

AGNT

ATNT

ACNT



FIG. 8B



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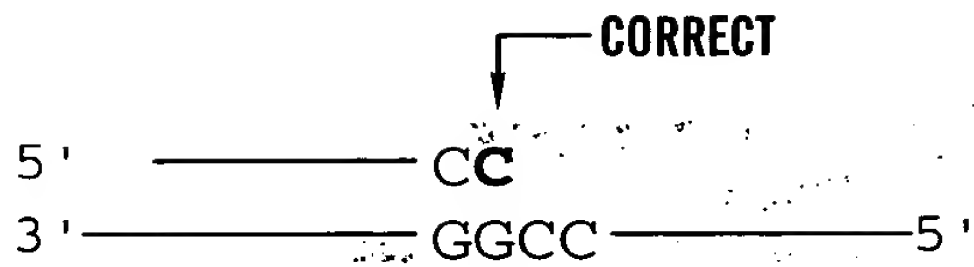


FIG. 9A

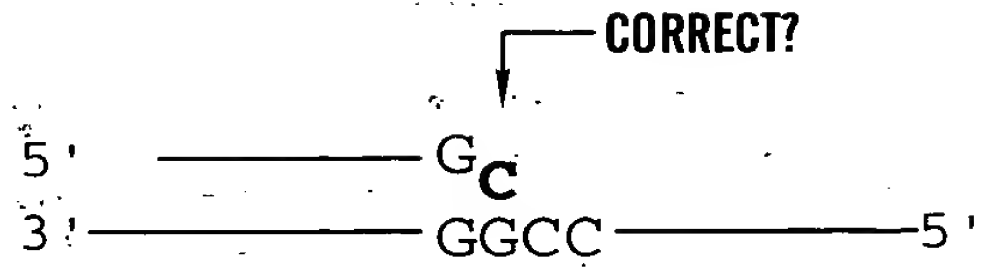


FIG. 9E

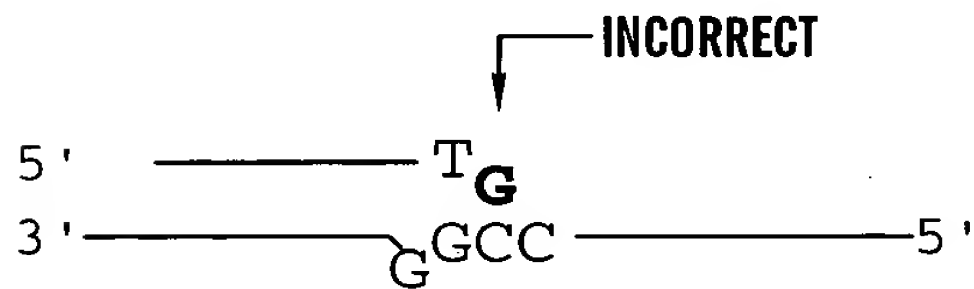


FIG. 9B

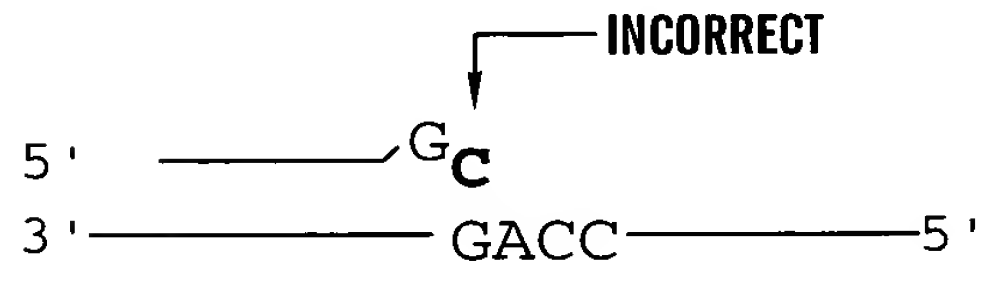


FIG. 9F

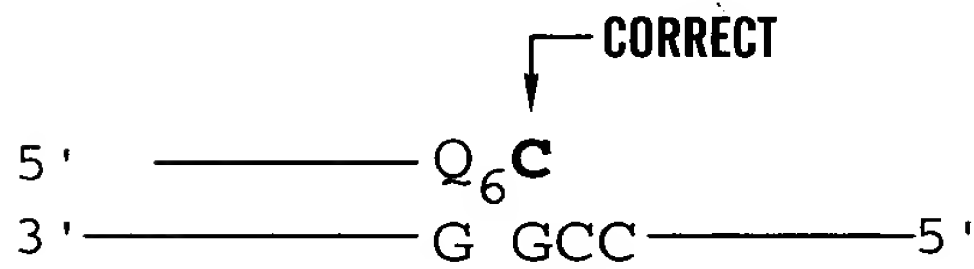


FIG. 9C

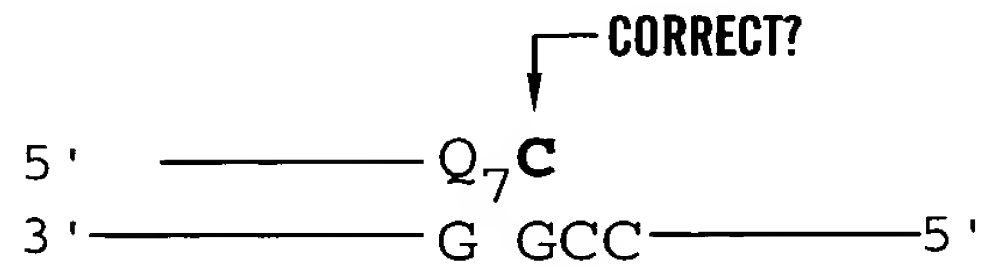


FIG. 9G

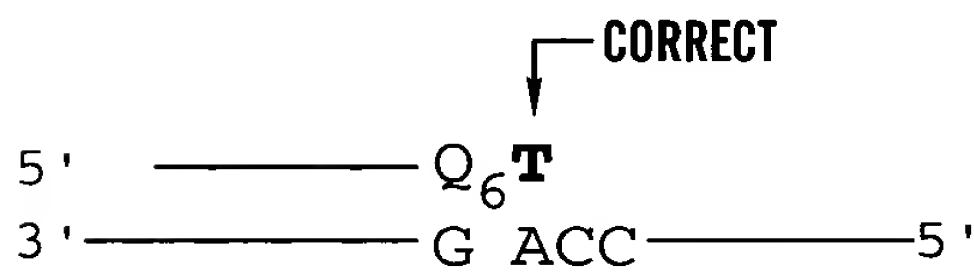


FIG. 9D

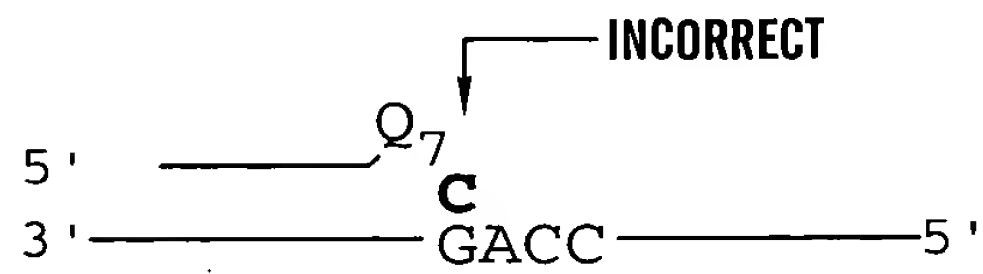


FIG. 9H



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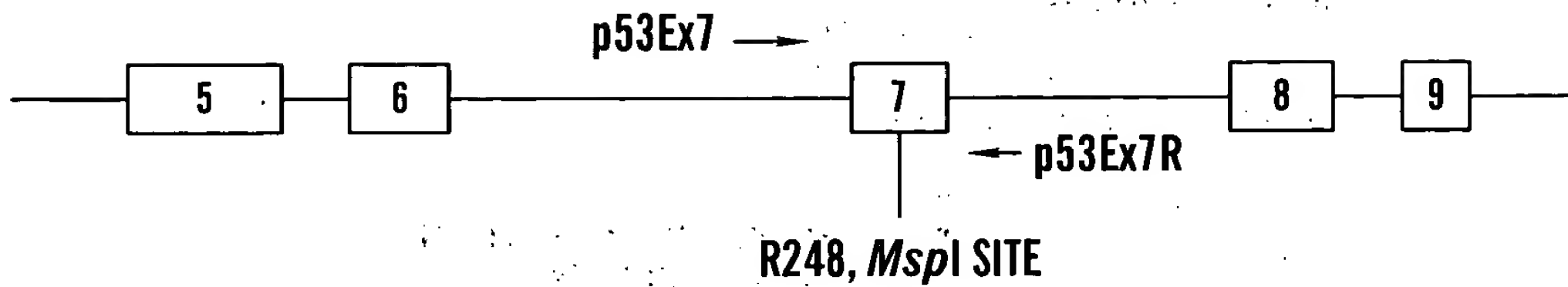


FIG. 10A

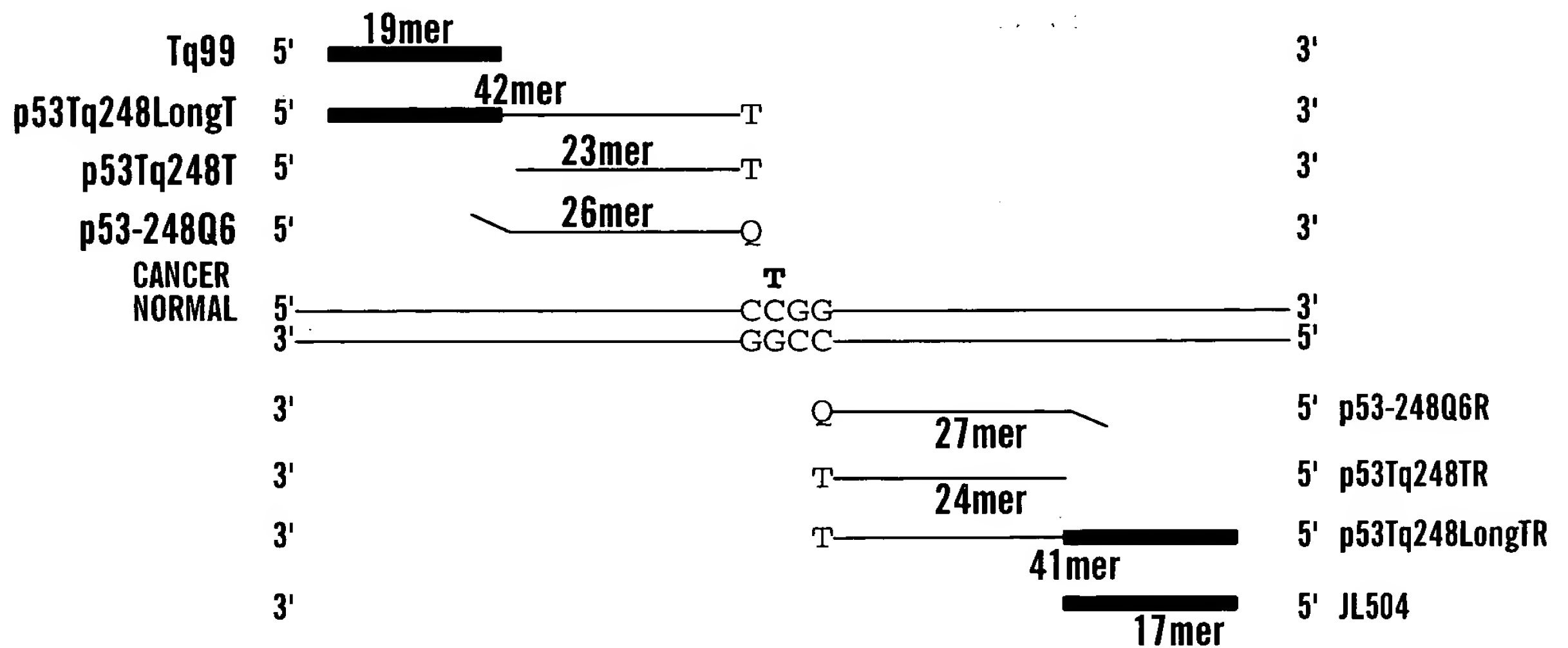
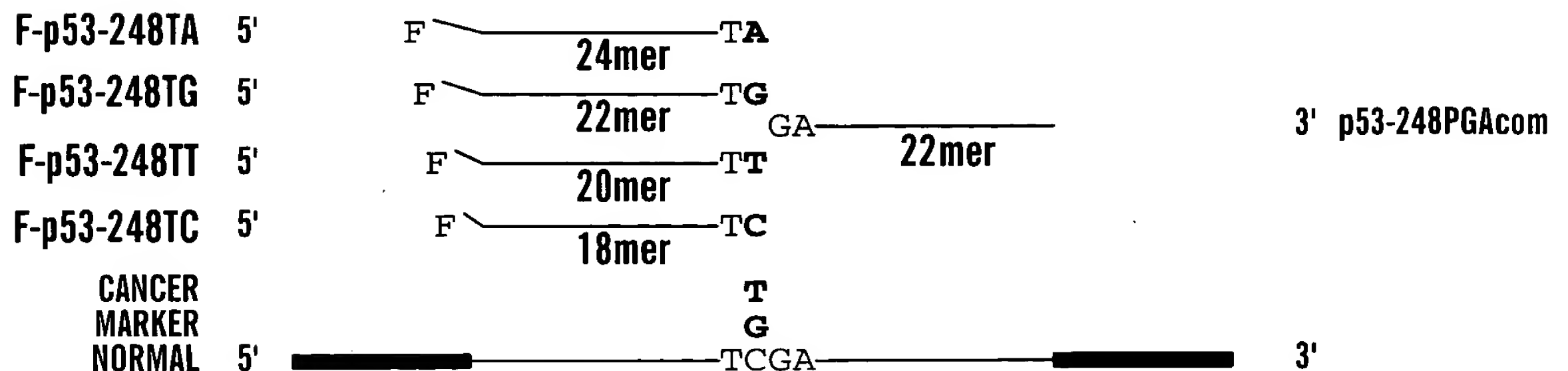
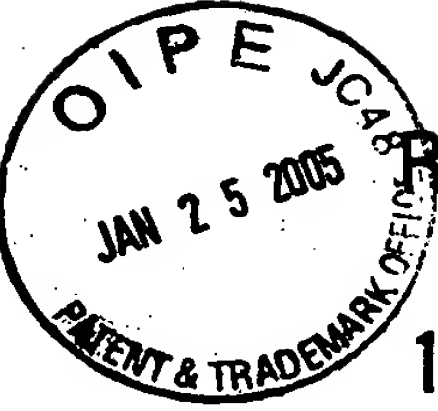


FIG. 10B



CODON 248 CONVERTED TO A *TaqI* SITE

FIG. 10C



REPLACEMENT SHEET

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MspI (CCGG)
codon 248
↓

PRIMERS

Ztop CTT GGA CGA GTT CAT ACG C

p53zip248short CTT GGA CGA GTT CAT ACG CGT TCC TGC ATG GGC GGC ATG A

p53-248short GT TCC TGC ATG GGC GGC A → pol
|| ||| ||| ||| ||| ||| ||| |

p53 exon 7 3' ... CA AGG AGC TAC CCG CCG TAC TTG GCG TCC GGG TAG GAG TGG TAG TAG TGT ... 5' (-)

PCR product

(MK not shown) 5' ... GT TCC TGC ATG GGC GGC ATG AAC CCG AGG AGG CCC ATC CTC ACC ATC ATC ACA ... 3' (+)
| ||| ||| ||| ||| ||| ||| ||| |||

p53-248short pol → GG TAG GAG TGG TAG TAG TG

p53zip248shortR C GGG TAG GAG TGG TAG TAG TGC ACC GCT GGG TCA AAC G

Zbot C ACC GCT GGG TCA AAC G

FIG. 11A

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*Msp*I (CCGG)
codon 248

[illegible]

FIG. 11B

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DISCRIMINATION

COMMON

P53LDR248FTCL

F-AAAAAAAA GC ATG GGC GGC ATG AAT C

P53LDR248FCA

F-AAAAAA GC ATG GGC GGC ATG AAC AAC A

P53LDR248FCG

F-AAAA GC ATG GGC GGC ATG AAC G

P53LDR248FCT

F-AA GC ATG GGC GGC ATG AAC T

P53LDR248FCC

F- GC ATG GGC GGC ATG AAC C₇...Ligase

P53LDR248PGG

GG AGG CCC ATC CTC ACC ATC ATC AT - block

3' (- strand)

5

CONVERSION

...GTA TGC GCA AGG AGC AGC TAC CCG CCG TAC TTG NGG TCC GGG TAG GAG TGG TAG TAG TGA ACC ...

PRODUCTS

FIG. 11C



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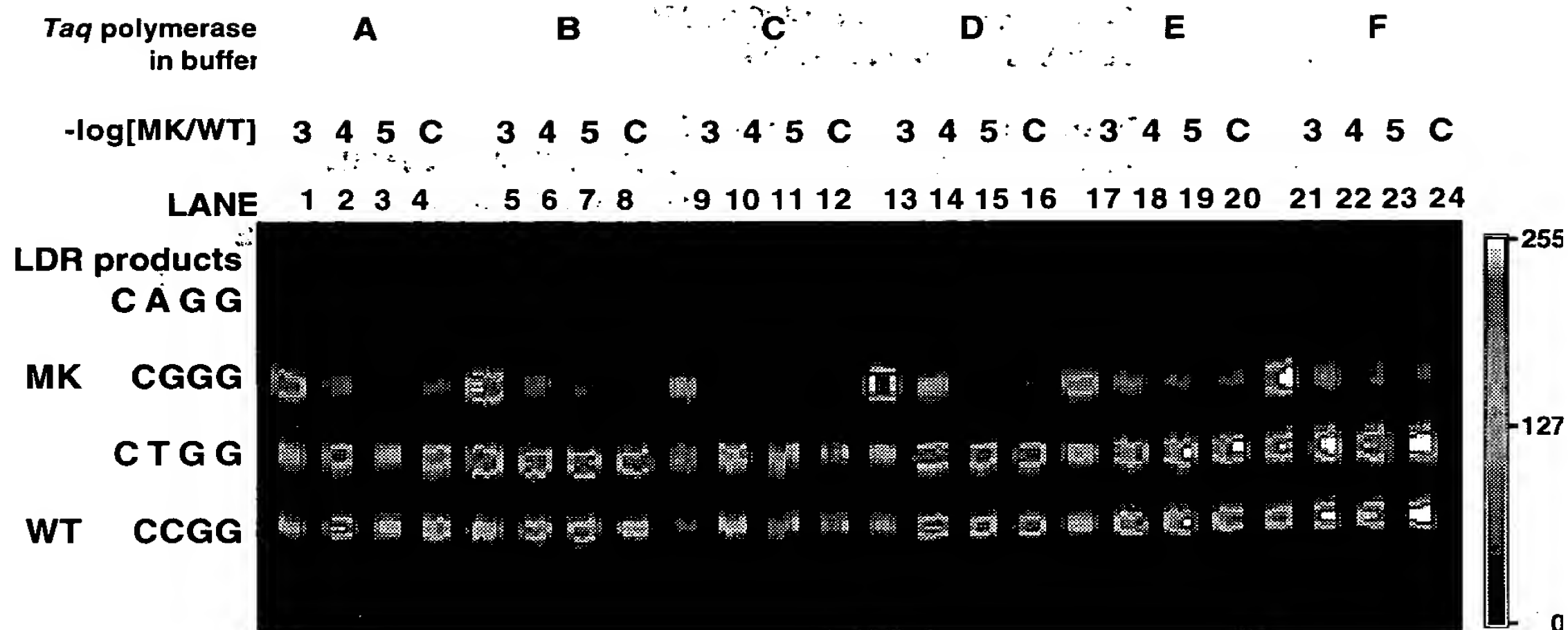


FIG. 12A

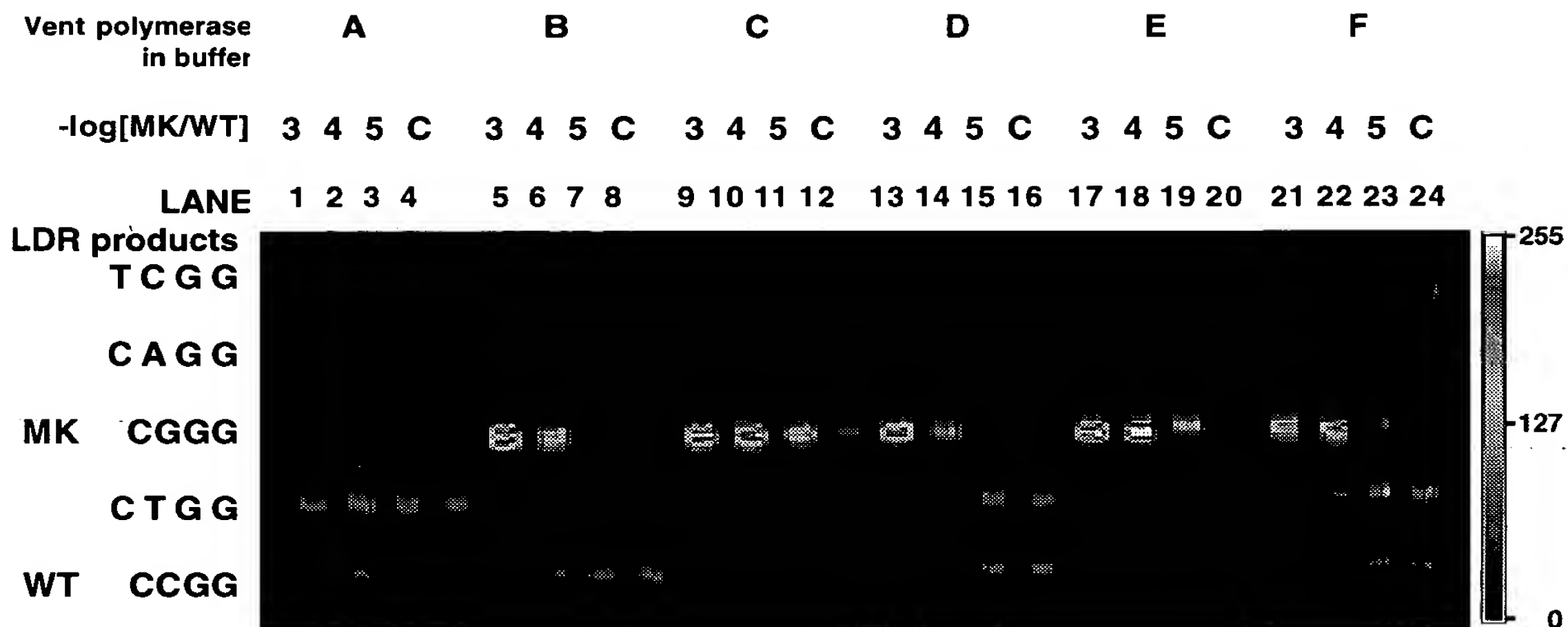
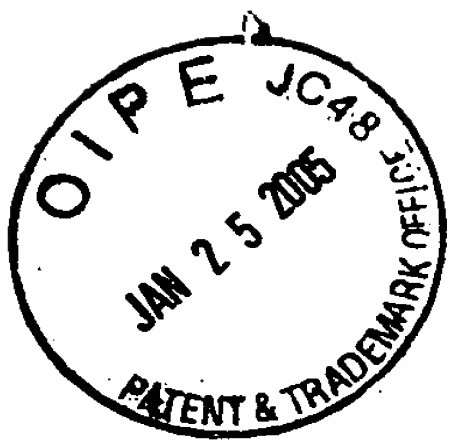


FIG. 12B



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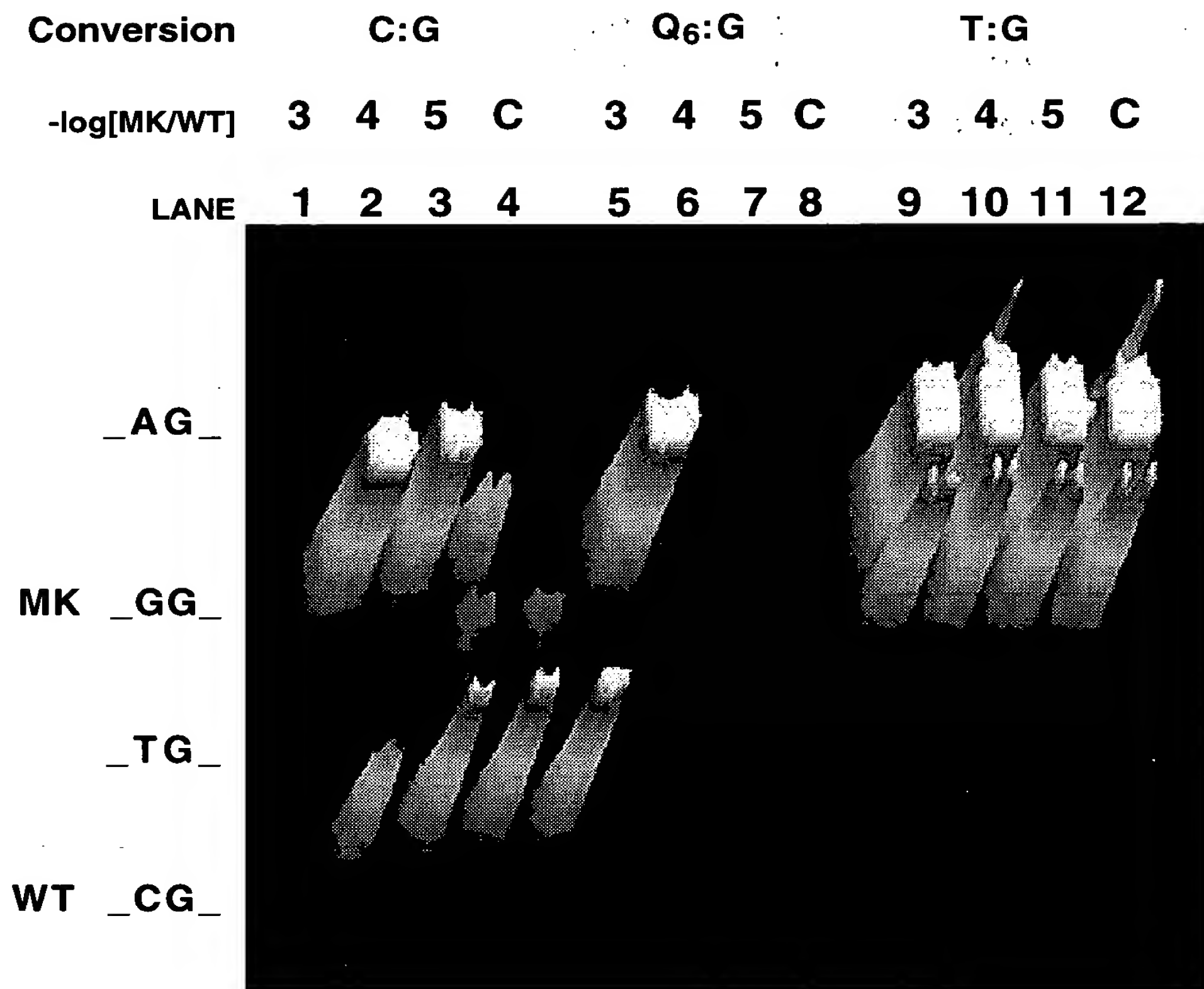


FIG. 13

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